NEUROSCIENCE

BRAIN DRAIN

AN INTERNAL PLUMBING SYSTEM RIDS THE BRAIN OF TOXIC WASTES. SLEEP IS WHEN THIS CLEANUP RITUAL OCCURS

By Maiken Nedergaard and Steven A. Goldman
The human brain weighs only about three pounds, or roughly 2 percent of the average adult body mass. Yet its cells consume 20 to 25 percent of the body's total energy. In the process, inordinate amounts of potentially toxic protein wastes and biological debris are generated. Each day, the adult brain eliminates a quarter of an ounce of worn-out proteins that must be replaced with newly made ones, a figure that translates into the replacement of half a pound of detritus a month and three pounds, the brain's own weight, over the course of a year.

To survive, the brain must have some way of flushing out debris. It is inconceivable that an organ so finely tuned to producing thoughts and actions would lack an efficient waste-disposal system. But until quite recently, the brain's plumbing system remained mysterious in several ways. Questions persisted as to what extraneous brain cells processed their own wastes or whether they might be transported out of the nervous system for disposal. And why is it that evolution did not seem to have made brains adept at delivering wastes to other organs in the body that are more specialized for removing debris? The liver, after all, is a powerhouse for disposing of or recycling waste products.

About five years ago we began trying to clarify how the brain eliminates proteins and other wastes. We also began to explore how interference with that process might cause the cognitive problems encountered in neurodegenerative disease. We thought that disturbances in waste clearance could contribute to such disorders because the disruption would be expected to lead to the accumulation of protein debris in and around cells.

This idea intrigued us because it was already known that such protein clumps, or aggregates, do indeed form in brain cells, most often in association with neurodegenerative disorders. What is more, it was known that the aggregates could impede the transmission of electrical and chemical signals in the brain and cause irreparable harm. In fact, the pathology of Alzheimer's, Parkinson's and other neurodegenerative diseases of aging can be reproduced in animal models by the forced overproduction of these protein aggregates.

In our research, we found an undiscovered system for clearing proteins and other wastes from the brain—and learned that this system is most active during sleep. The need to remove potentially toxic wastes from the brain may, in fact, help explain the mystery of why we sleep and hence retreat from wakefulness for a third of our lives. We fully expect that an understanding of what happens when this system malfunctions will lead us to both new diagnostic techniques and treatments for a host of neurodegenerative illnesses.

The Lymphatic System
In more regions of the body, a network of intricate fluid-carrying vessels, known as the lymphatic system, eliminates protein waste from tissues. Waste-carrying fluid moves throughout this network between cells. The fluid collects into small ducts that then lead to larger ones and eventually into blood vessels. This duct structure also provides a path for immune defense, because lymph nodes, a repository of infection-fighting white blood cells, populate ducts at key points throughout the network. Yet for a century neuroscientists had believed that the lymphatic system did not exist in the brain or spinal cord. The prevailing view held that the brain eliminated wastes on its own. Our research suggests that this is not the complete story.

The brain's blood vessels are surrounded by what are called perivascular spaces. They are doughnut-shaped tunnels that surround every vessel. The inner wall of each space is made of the surface of vascular cells, mostly endothelial cells and smooth

IN BRIEF
Every day the brain eliminates a quarter of an ounce of worn-out proteins that must be replaced with newly made ones. The waste disposal process reallocated a pound of debris a month and three pounds a year, equivalent to the brain's own weight.

Where do these wastes go if the brain lacks the elaborate network of lymph vessels that transports wastes outside the nervous system? New research has recently found a ductus-carrying passage in the brain that are most active during sleep.

The lymphatic system, as these fluid vessels are known, may become a critical target for the treatment of neurodegenerative diseases such as Alzheimer's or Parkinson's that result from the buildup of toxic proteins that are not cleared from the brain.
Clearing the Head

An intricate system of vessels—the glymphatic system—snakes throughout the brain, carrying fluid that aids the brain’s waste removal processes. This fluid, known as cerebrospinal fluid (CSF), is produced in the ventricles of the brain and drains into the subarachnoid space, where it mixes with other cerebrospinal fluids from the spinal cord. The CSF then flows through the subarachnoid space, collecting and removing waste products from the brain tissue.

**Incoming Fluid**

Cerebrospinal fluid (CSF) from the subarachnoid space enters the skull and brain through the subarachnoid space, where it collects metabolic waste products and transport molecules. Once in the subarachnoid space, the CSF flows through the ventricles of the brain, where it mixes with other cerebrospinal fluids from the spinal cord.

**Outgoing Wastes**

The fluid, having picked up waste from brain tissue, is transported back to the ventricles, where it is reabsorbed, forming a continuous circulation that removes waste from the brain tissue. The waste products are then transported into the lymphatic system and eventually the bloodstream.

Illustration by Julianne K. Sied

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In the healthy brain, the lymphatic system clears proteins associated with Alzheimer’s, Parkinson’s and other neurological diseases.

perivascular space and into the astrocytes, thereby gaining ac-

cess to the brain tissue.

We asked whether the perivascular space might constitute a lymphatic system. Could it perhaps provide a conduit for cerebral fluid? Atrial pulsations might drive the CSF through the perivascular space. From there, some of it could enter astrocytes through their end foot. It could then move into the area between cells and finally to the perivascular space around veins to clear waste products from the brain.

Along with our laboratory colleagues Jeff Hilt and Bashir Dzau, we went onto confirm this hypothesis. Using chemical dyes that stained the fluid, combined with microscopic tech-
niques that allowed us to image deep inside live brain tissue, we could directly observe that the pumping of blood propelled large quantities of CSF into the perivascular space surrounding arteries. Using astrocytes as tracers, the CSF then moved through the brain tissue, where itthe astrology and picked up interdisciplinary.

The fluids exited the brain through the perivascular space that surrounded small veins draining the brain, and these veins in turn merged into larger ones that continued into the neck. The waste liquids were on to enter the lymph system, from which they flowed back into the general blood circulation. They combined there with protein waste products from other organs that were ultimately destined for filtering by the kidneys or processing by the liver.

When we began our research, we had no idea that astrocytes played such a critical role in the brain’s downstream lymphatic system. Additional proof came when we used genetically engineered mice that lacked the aquaporin-4 protein that makes up the astrocytes’ water channels. The rate of CSF flow entering the astrocytes dropped by 60 percent, greatly slowing fluid transport through their brain.

We had now traced a complete pathway within the brain for these cleansing fluids to effectively sweep away waste products. We named our discovery the lymphatic system. The newly coined word combined the words “gill”—a type of brain cell of which the astrocyte is one example—and “lymphatic,” this reference this newly discovered function of the brain’s glial cells. As we came to recognize the important role of the lymphatic sys-tem, we immediately wondered whether proteins that build up in the brain in neurodegenerative diseases might, in the healthy brain, be typically washed out along with other, more mundane cellular waste. In particular, we focused on a protein linked to Alzheimer’s called beta-amyloid, which had previously been thought to be cleared under normal circumstances by degradation or recycling processes that take place within all brain cells. In Alzheimer’s, aggregates of beta-amyloid form plaques between cells that may contribute to the disease process. We found that in a healthy brain, beta-amyloid is cleared by the lymphatic system. Other proteins implicated in neurodegenerative diseases, such as the synuclein proteins that turn up in Parkinson’s, Lewy body disease and multisystem atrophy, might also be carried away and could build up abnormally if the lymphatic system were to malfunction.

A symptom that accompanies Alzheimer’s and other neurodegenerative diseases provided a hint of how to proceed. Many patients with Alzheimer’s experience sleep dis-

turbances long before their dementia becomes apparent. In 

older individuals, sleep becomes more fragmented and shallow 

and lasts a shorter time. Epidemiological studies have shown that patients who reported poor sleep in middle life were at greater risk for cognitive decline than control subjects when tested 25 years later.

Even healthy individuals who are forced to stay awake either 

symptoms more typical of neurodegenerative disease and mental ill-

ness—poor concentration, memory lapses, fatigue, irritability, and emotional ups and downs. Profound sleep deprivation may 

produce confusion and hallucinations, potentially leading to 

epileptic seizures and even death. Indeed, lab animals may die 

when deprived of sleep for as little as several days, and humans 

are no more resilient. In humans, familial insomnia is an 

inherited disease that causes patients to sleep progressively less 

until they die, usually within 18 months of diagnosis.

Knowing all this, we speculated that sleep difficulties of dementia might not just be a side effect of the disorder but 

might contribute to the disease process itself. Moreover, if the lymphatic system cleared beta-amyloid during sleep at a higher rate than when awake, perhaps the poor sleeping patterns of patients with neurodegenerative disorders might contribute to a worsening of the disease. Because our initial experiments had been performed on anesthetized mice, we further speculated that the fast flow that we noted were not necessarily what we might anticipate to an awake and active brain, which would be subject to other demands in its typical functional.

To test the idea, Lulu Xie and Yong Kang, both in the Ne-

A.F., in the American online.
erated Laboratory, trained mice to see and still underwent a micro-
scope to capture images of a tracer chemical in the CIS using a
novel imaging technique called two-photon microscopy. We com-
pared how the tracer moved through the glialytic system in
awake versus sleeping mice. Because Imaging is neither invasive
nor painful, thics mice remain quiet and compliant, so much so
that animals often fall asleep without being imaged. We were thus
able to image infusions of CI5 in a particular area of the same
mouse brain during both sleep and wakefulness.

CIB in the glialytic system, it turned out, felt dramatically
less similar, the study mice were awake. Within minutes after the onset
of sleep or the effects of anesthesia, however, infusions of the fluid
increased significantly. In collaboration with Charles Nicols-
son of New York University, we found that the brain's "intentional
space"—the area between cells through which glialytic fluid
flows on its way to perivascular spaces around bloodvessels—rose by
more than 40 percent when mice fell asleep. We now believe that
the flow of glialytic fluid increases during sleep because the
space between the cells expands, which helps to push fluid
through the brain tissue.

Our research also revealed how the rate of fluid flow is con-
trolled. A neurotransmitter, or signaling molecule, called nor-
ephrine appeared to regulate the volume of the interstitial
area and consequently the flow of glialytic fluid. Levels of
noradrenaline rose when mice were awake and were scarce
during sleep, implying that transient, sleep-related dips in nor-
ephrine availability led to enhanced glialytic flow.

THE POWER OF SLEEP

Noradrenalin means what it says: the expression and contraction of the
interstitial space during sleep was important to both brain
function and protein-waste clearance, we then wanted to test a
contingency by this observation: Could sleep depuration precipi-
tate neurodegenerative disease? Experiments that we conduct-
ed in mice showed that during sleep, the glialytic system did
indeed remove beta-amyloid from the brain with remarkable
efficiency: its clearance rate more than doubled with sleep. On
the other hand, mice genetically engineered so that they lacked
aquaporin-4 water channels in astrocytes demonstrated mark-
edly impaired glialytic fluxes—clearing 40 percent less beta-
amyloid than control animals.

The remarkably high percentage of beta-amyloid removed
challenged the widely held idea that brain cells break down all
their own wastes internally; through degradation processes
called autophagy and microautophagy, now we know that the
brain removes a good deal of unwanted proteins whole, sweep-
ing them out for later degradation. These new findings, more-
over, seemed to confirm that the sleeping brain exports protein
waste, including beta-amyloid, through the glialytic trans- port
system. Additional support for this thesis came from Da-
vil M. Holmogar's group at Washington University in St. Louis,
which demonstrated that beta-amyloid concentration in the
interstitial space is higher during wakefulness than in sleep and
that sleep deprivation aggravates amyloid-40 plaque formation in
mice genetically engineered to accumulate it in excess.

So far these investigations have not moved beyond basic re-
search labs. Drug companies have yet to consider antidiabetes
therapies that would physically remove amyloid and other tox-
ic proteins by washing out the brain with glialytic fluids

But maybe they should. New strategies are desperately needed
for a disease that costs the U.S. health care system $220 billion
annually. A number of clinical trials for Alzheimer's are under
way, although no drug in development has yet demonstrated a
clear-cut benefit. Stimulation glialytic flows offers a new ap-
proach that is worth investigating.

A pharmaceuticals that regulates the glialytic system by
increasing the rate of flow during sleep could literally flush
amyloid out of the brain. A treatment used for a well-known
neurological syndrome provides a clue that this approach
might work. Nonnal mice have a high level of beta-am-
LYoid typically seen in the elderly, in a form of dementia in which ex-
cessive CI5 accumulates in the hollow central brain cavities, the
Cerebral ventricles. When a procedure called lumbar puncture
removes the fluid by draining it out, patients often exhibit re-
markable improvements in their cognitive abilities. The basis
for this observation has long been a mystery. Our research sug-
gests that restoring fluid flows through the glialytic system
might mediate the restoration of cognition in these patients.

Even if a new drug is not imminent, knowledge of the glial-
ytic system suggests fresh ideas for diagnosing Alzheimer's and
other neurological conditions. A recent study by Helen
Roosevelt of the Stony Brook School of Medicine has shown
that standard magnetic resonance imaging can visualize and
quantify the activity of the glialytic system. The technology
may allow tests of glialytic fluid flow designed to predict disease
progression in patients suffering from Alzheimer's or related
dementias or normal-pressure hydrocephalus. It might even fore-
tell the ability of patients with traumatic brain injuries to recov-
er. Most of our studies of the glialytic system to date have fo-
cused on the removal of protein wastes. Yet the glialytic
system may also prove to be a fertile area for gaining a basic un-
derstanding of how the brain works.

Intriguingly, fluids moving through the glialytic system
may do more than remove wastes. They may deliver a variety of
nutrients and other small yet large to brain tissue. A new study showed
that glialytic channels deliver glucose to neurons to provide
energy. Further studies are now investigating whether white
matter, the insulating sheath surrounding axons' wirelike
extensions, called axons, may rely on the glialytic system for
delivery of both nutrients and materials needed for maintain-
ing the cellular structural integrity. Such studies promise to eluci-
date the many unexpected roles of the glialytic system in
the daily life—and nighttime—of the brain.

MORE TO EXPLORE

Distinct Functional State of Amytrophic Lateral Sclerosis
Asymmetry During Sleep and Wakefulness

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